Amendments to the Drawings:

The attached sheet of drawings are renumbered sheets, in which original sheets of figures 1- 241 are placed with sheets 1-138. In the Figures, Figs. 1, 2, 4, 5, 7-12, 14, 15, 17-24, 26-29, 31-36, 38-45, 47-56, 58, 59, 61, 62, 64, 65, 67, 68, 70, 71, 73, 74, 76, 77, 79, 80, 82-87, 89, 90, 92, 93, 95-100, 102, 103, 106, 107, 110, 111, 114, 115, 118, 119, 122, and 123 have been deleted, and figures have been renumbered as follows:

Old number	New Number
3	1
6	2
13	3
16	4
25	5
30	6
37	7
46	8
57	9
60	10
63	11
66	12
69	13
72	14
75	15
78	16
81	17
88	18
91	19
94	20
101	21
104	22
105	23
108	24
109	25
112	26
113	27
116	28
117	29
120	30
121	31
124	32
125	33
126	34

127	35
128	36
129	37
130	38
131	39
132	40

Attachment: Replacement Sheet

REMARKS/ARGUMENTS

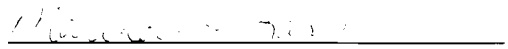
This preliminary amendment is made in partial response to the Notification of Missing Requirements mailed to June 9, 2006. In order to reduce the fees that are due, drawings which are reflected duplicated in the sequence listing have been deleted and a new set of renumbered drawing sheets has been provided. The specification has been amended to conform to the renumbered drawings, and the claims have been amended to delete reference to figure numbers.

To facilitate publication and counting of pages for fee purposes, a substitute specification is filed herewith. The difference between this substitute specification and the specification as filed are reflected in the marked up copies of the specification attached hereto.

The revised page count for the application, as amended is 106 pages less. As a result, the extra page fee due is for 205 pages, not 311 as indicated in the notice, and the fee due is reduced to $1380.

In addition, Applicants request an extension of time sufficient to make this response timely and enclose the appropriate fee.

Respectfully submitted,



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By "operatively linked" in respect of a regulatory element, nucleic acid or nucleic acid fragment and terminator, is meant that the regulatory element is capable of causing expression of said nucleic acid or nucleic acid fragment in a plant cell and said terminator is capable of terminating expression of said nucleic acid or nucleic acid fragment in a plant cell. Preferably, said regulatory element is upstream of said nucleic acid or nucleic acid fragment and said terminator is downstream of said nucleic acid or nucleic acid fragment.

By "an effective amount" of a nucleic acid or nucleic acid fragment is meant an amount sufficient to result in an identifiable phenotypic trait in said plant, or a plant, plant seed or other plant part derived therefrom. Such amounts can be readily determined by an appropriately skilled person, taking into account the type of plant, the route of administration and other relevant factors. Such a person will readily be able to determine a suitable amount and method of administration. See, for example, Maniatis et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, the entire disclosure of which is incorporated herein by reference.

It will also be understood that the term "comprises" (or its grammatical variants) as used in this specification is equivalent to the term "includes" and should not be taken as excluding the presence of other elements or features.

Such nucleic acids or nucleic acid fragments could be assembled to form a consensus contig. As used herein, the term "consensus contig" refers to a nucleotide sequence that is assembled from two or more constituent nucleotide sequences that share common or overlapping regions of sequence homology. For example, the nucleotide sequence of two or more nucleic acids or nucleic acid fragments can be compared and aligned in order to identify common or overlapping sequences. Where common or overlapping sequences exist between two or more nucleic acids or nucleic acid fragments, the sequences (and thus their corresponding nucleic acids or nucleic acid fragments) can be assembled into a single contiguous nucleotide sequence.

In a preferred embodiment of this aspect of the invention, the substantially purified or isolated nucleic acid or nucleic acid fragment encodes a CS or CS-like polypeptide and including a nucleotide sequence selected from the group consisting of (a) sequences shown in ~~Figures 1,3, 4,6, 7,9, 99,101, 102,104,~~

~~114,118 and 122 hereto (~~SEQ ID NOS 1, 3 to 10,11,13 to 16, 17,19, 327,329 to 335,336, 338 to 344,349, 351 and, 353 ~~respectively)~~; (b) complements of the sequences recited in (a); (c) sequences antisense to the sequences recited in (a) and (b); (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c); and (e) RNA sequences corresponding to the sequences recited in (a), (b), (c) and (d).

In a further preferred embodiment of this aspect of the invention, the substantially purified or isolated nucleic acid or nucleic acid fragment encodes a MDH or MDH-like polypeptide and including a nucleotide sequence selected from the group consisting of (a) sequence shown in ~~Figures 11,13, 14,16, 17,19, 21, 23,25, 26,28, 30,31, 33,35, 37,38, 40,55, 57,58, 60,61, 63,64, 66,67, 69,70, 72,73, 75,76, 78,79, 81,82 and 84 hereto (~~SEQ ID NOS. 21,23 to 29, 30,32 to 33,34, 36,38, 40,42 to 43,44, 46,48 to 110,111, 113,115, 117 to 182,183, 185,205, 207 to 217,218, 220 to 251,252, 254 to 270,271, 273 to 275,276, 278 to 287,288, 290 to 292,293, 295 to 296,297, 299 to 301,304 to 305,306 and, ~~308~~); (b) complements of the sequences recited in (a); (c) sequences antisense to the sequences recited in (a) and (b); (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c); and (e) RNA sequences corresponding to the sequences recited in (a), (b), (c) and (d).

In a further preferred embodiment of this aspect of the invention, the substantially purified or isolated nucleic acid or nucleic acid fragment encodes a PEPC or PEPC-like polypeptide and including a nucleotide sequence selected from the group consisting of (a) sequences shown in ~~Figures 42,44, 46,47, 49, 51,53, 86,88, 89,91, 92,94, 95,97 and 110 hereto (~~SEQ ID NOS 187, 189,191 to 197,199, 201,203, 310,312 to 314,315, 317 to 318,319, 321 to 322,323, 325 and 347 ~~respectively)~~; (b) complements of the sequences recited in (a); (c) sequences antisense to the sequences recited in (a) and (b); (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c); and (e) RNA sequences corresponding to the sequences recited in (a), (b), (c) and (d).

Nucleic acids or nucleic acid fragments encoding at least a portion of several CS, MDH and PEPC polypeptides have been isolated and identified. Genes encoding other CS orCS-like, MDH orMDH-like and PEPC or PEPC-like proteins, either as cDNAs or genomic DNAs, may be isolated directly by using all

ryegrass (*Lolium*) or fescue (*Festuca*) species, selected from the group consisting of CS or CS-like, MDH orMDH-like and PEPC or PEPC-like polypeptides ; and functionally active fragments and variants of these polypeptides.

The clover (*Trifolium*), medic (*Medicago*), ryegrass (*Lolium*) or fescue (*Festuca*) species may be of any suitable type, including white clover (*Trifolium repens*), red clover (*Trifolium pratense*), subterranean clover (*Trifoliumsubterraneum*), alfalfa (*Medicago sativa*), Italian or annual ryegrass (*Lolium multiflorum*), perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinacea*), meadow fescue (*Festuca pratensis*) and red fescue (*Festuca rubra*). Preferably the species is a clover or a ryegrass, more preferably white clover (*T. repens*) or perennial ryegrass (*L. perenne*).

In a preferred embodiment of this aspect of the invention, the substantially purified or isolated CS or CS-like polypeptide includes an amino acid sequence selected from the group consisting of sequences shown in ~~Figures 2,5, 8,10, 100, 103,115, 119,123 hereto (~~SEQ ID NOS 2,12, 18,20, 328,337, 350,352 and 354 ~~respectively)~~; and functionally active fragments and variants thereof.

In a further preferred embodiment of this aspect of the invention, the substantially purified or isolated MDH orMDH-like polypeptide includes an amino acid sequence selected from the group consisting of sequences shown in ~~Figures 12,15, 18,20, 22,24, 27,29, 32,34, 36,39, 41,56, 59,62, 65,68, 71,74, 77,80, 83 and 85 hereto (~~SEQ ID NOS 22,31, 35,37, 39,41, 45,47, 112,114, 116,184, 186,206, 219,253, 272,277, 289,294, 297,303, 307 and 309~~, respectively)~~; and functionally active fragments and variants thereof.

In a further preferred embodiment of this aspect of the invention, the substantially purified or isolated PEPC or PEPC-like polypeptide includes an amino acid sequence selected from the group consisting of sequences shown in ~~Figures 43,45, 48,50, 52,54, 87,90, 93,96, 98 and 111 hereto (~~SEQ ID NOS 188,190, 198,200, 202,204, 311,316, 320,324, 326, and 348~~, respectively)~~; and functionally active fragments and variants thereof.

In a further embodiment of this aspect of the invention, there is provided a polypeptide produced (e. g. recombinantly) from a nucleic acid or nucleic acid

as pGEM-T Easy vector sequence up to the EcoRI cut site both at the 5' and 3' end.

Plasmid maps and the full DNA sequences of TrCSa, TrCSb, TrCSd, TrMDH and TrPEPC polypeptides were obtained ~~(Figures 1, 2, 5, 6, 9, 10, 13, 14, 17,18, 21,22, 25,26, 29 and 30)~~ (SEQ ID NOS: 1, 2, 12, Figure 2, SEQ ID NOS: 19, 20, Figure 3, SEQ ID NOS: 30, 34, 35, 38, 39, Figure 5, SEQ ID NOS: 44, 47 and Figure 6).

TABLE 3

List of primers used for sequencing of the full-length cDNAs encoding CS, MDH and PEPC

gene name	clone ID	sequencing primer	primer sequence (5' > 3')
TrCSa	05wc1HsB08	05wc1HsB08.f1	TTGCCCGAGGCTATACTGTGGC
		05wc1HsB08.f2	CAGCTCACCTAGTTGCTAG
		05wc1HsB08.f3	CCATGGCCTAATGTTGATGC
		05wc1HsB08.r1	TTGGCCTTTCAAGTGGCATTCC
		05wc1HsB08.r2	CAGAATGGGAGGCACGACTTC
		05wc1HsB08.r3	ATGTGAGCATAGTTTGCACC
TrCSb	05wc2HsD09	05wc2HsD09.f1	GACTGCCAGAAAACACTTCCAGG
		05wc2HsDO9.f2	ATGACTGCTTTAGTGTGG
		05wc2HsD09. r1	CTCAAGTTTCTCCAGTGTGACAC
		05wc2HsDO9.r2	TGACTTATGTATCCCACC
		05wc2HsD09.r3	GCTCTGAATGGTTTAGCTGG
TrCSd	10wc1BsF10	10wc1BsF10.f1	GCACTGCCTGTTTCTGCTCATCC
		10wc1BsF10.f2	AGCCAACTTATGAGGATAGC
		10wc1BsF10.r1	CTCCAATACTCCTCGCGACGCC
		10wc1BsF10.r2	AGGCACAACCTGGCCACTG
		10wc1BsF10.r3	ACGTTGCCACCTTCATGATC
TrMDH	13wc1 NsD01	13wc1NsD01.f1	GTTGTTATACCTGCTGGTGTT
		13wc1NsD01.r1	CTCACTCAACCCTTGGAGAT
TrPEPC	15wc1DsH12	15wc1DsH12.f1	TCCTAAGAAACTTGAAGAGCTCGG
		15wc1DsH12.f2	AGATGTTTGCTTACTAGC
		15wc1DsH12.r1	GCCAGCAGCAATACCCTTCATGG
		15wc1DsH12.r2	TTGCTTCTCAACTGTTCC

TABLE 3

List of primers used for sequencing of the full-length cDNAs encoding CS, MDH and PEPC

Column Inserted

gene name	clone ID	sequencing primer	Seq. ID NO:	primer sequence (5'>3')
TrCSa	05wc1HsB08	05wc1HsB08.f1	355	TTGCCCGAGGCTATACTGTGGC
		05wc1HsB08.f2	356	CAGCTCACCTAGTTGCTAG
		05wc1HsB08.f3	357	CCATGGCCTAATGTTGATGC
		05wc1HsB08.r1	358	TTGGCCTTTCAAGTGGCATTCC
		05wc1HsB08.r2	359	CAGAATGGGAGGCACGACTTC
		05wc1HsB08.r3	360	ATGTGAGCATAGTTTGCACC
TrCSb	05wc2HsD09	05wc2HsD09.f1	361	GACTGCCAGAAAACACTTCCAGG
		05wc2HsD09.f2	362	ATGACTGCTTTAGTGTGG
		05wc2HsD09.r1	363	CTCAAGTTTCTCCAGTGTGACAC
		05wc2HsD09.r2	364	TGACTTATGTATCCCACC
		05wc2HsD09.r3	365	GCTCTGAATGGTTTAGCTGG
TrCSd	10wc1BsF10	10wc1BsF10.f1	366	GCACTGCCTGTTTCTGCTCATCC
		10wc1BsF10.f2	367	AGCCAACTTATGAGGATAGC
		10wc1BsF10.r1	368	CTCCAATACTCCTCGCGACGCC
		10wc1BsF10.r2	369	AGGCACAACCTGGCCACTG
		10wc1BsF10.r3	370	ACGTTGCCACCTTCATGATC
TrMDH	13wc1NsD01	13wc1NsD01.f1	371	GTTGTTATACCTGCTGGTGTT
		13wc1NsD01.r1	372	CTCACTCAACCCTTGGAGAT
TrPEPC	15wc1DsH12	15wc1DsH12.f1	373	TCCTAAGAAACTTGAAGAGCTCGG
		15wc1DsH12.f2	374	AGATGTTTGCTTACTAGC
		15wc1DsH12.r1	375	GCCAGCAGCAATACCCTTCATGG
		15wc1DsH12.r2	376	TTGCTTCTCAACTGTTCC

EXAMPLE 4

Development of binary transformation vectors containing chimeric genes with cDNA sequences encoding CS, MDH and PEPC

To alter the expression of the polypeptides involved in organic acid biosynthesis to improve phosphorus acquisition efficiency as well as aluminium

The orientation of the constructs (sense or antisense) was checked by restriction enzyme digest and sequencing which also confirmed the correctness of the sequence. Transformation vectors containing chimeric genes using full-length open reading frame cDNAs encoding white clover TrCSa, TrCSb, TrCSd, TrMDH and TrPEPC proteins in sense orientation under the control of the CaMV35S2 promoter were generated ~~(Figures 4,8, 12,16, 20,24, 28 and 32).~~ (SEQ ID NOS: 11, 18, 22, Figure 4, SEQ ID NOS: 37, 41 and 46).

TABLE 4

List of primers used to PCR-amplify the open reading frames of cDNAs encoding CS, MDH and PEPC

Column unseated

gene name	clone ID	primer	Seq ID No	primer sequence (5'>3')
TrCSa	05wc1HsB08	05wc1HsB08f	377	GGGGACAAGTTTGTACAAAAAAGCAGGCTT GATCTTAATGGCGTTCTTTCG
		05wc1HsB08r	378	GGGGACCACTTTGTACAAGAAAGCTGGGTT TTCAATTTTAGGACGATGCG
TrCSb	05wc2HsD09	05wc2HsD09f	379	GGGGACAAGTTTGTACAAAAAAGCAGGCTT TGTTGATTGATCTTAATGGC
		05wc2HsD09r	380	GGGGACCACTTTGTACAAGAAAGCTGGGTT AGTAATCCACAGATAACCG
TrCSd	10wc1BsF10	10wc1BsF10f	381	GGGGACAAGTTTGTACAAAAAAGCAGGCTC TAGATTGTTGATTGATCTAAATGGC
		10wc1BsF10r	382	GGGGACCACTTTGTACAAGAAAGCTGGGTC TAGATTCAATTTTAGGATGATGCACC
TrMDH	13wc1NsD01	13wc1NsD01f	383	GGGGACAAGTTTGTACAAAAAAGCAGGCTC TAGAAATTCCCATTACCATTCATTCC
		13wc1NsD01r	384	GGGGACCACTTTGTACAAGAAAGCTGGGTC TAGATTGACATTCTCTCGCATGGACGC
TrPEPC	15wc1DsH12	15wc1DsH12f	385	GGGGACAAGTTTGTACAAAAAAGCAGGCTT GAGAAGGAGTGAATTGCTCC
		15wc1DsH12r	386	GGGGACCACTTTGTACAAGAAAGCTGGGTA TGATATCTTAGCACACACTTAAC

EXAMPLE 5

Development of binary transformation vectors containing chimeric genes with a combination of 2 or more cDNA sequences encoding CS, MDH and PEPC

To alter the expression of the polypeptides involved in organic acid biosynthesis to improve phosphorus acquisition efficiency as well as aluminium and acid soil tolerance in forage plants, a modular binary transformation vector system was used (Figure 33). The modular binary vector system enables simultaneous integration of up to seven transgenes the expression of which is controlled by individual promoter and terminator sequences into the plant genome (Goderis *et al.*, 2002).

The modular binary vector system consists of a pPZP200-derived vector (Hajdukiewicz *et al.*, 1994) backbone containing within the T-DNA a number of

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and acid soil tolerance in forage plants, a modular binary transformation vector system was used (Figure 12533). The modular binary vector system enables simultaneous integration of up to seven transgenes the expression of which is controlled by individual promoter and terminator sequences into the plant genome (Goderis et al., 2002).

The modular binary vector system consists of a pPZP200-derived vector (Hajdukiewicz etal., 1994) backbone containing within the T-DNA a number of unique restriction sites recognised by homingendonucleases. The same restriction sites are present in pUC18-based auxiliary vectors flanking standard multicloning sites. Expression cassettes comprising a selectable marker gene sequence or acDNA sequence to be introduced into the plant under the control of regulatory sequences like promoter and terminator can be constructed in the auxiliary vectors and then transferred to the binary vector backbone utilising the homing endonuclease restriction sites. Up to seven expression cassettes can thus be integrated into a single binary transformation vector. The system is highly flexible and allows for any combination ofcDNA sequence to be introduced into the plant with any regulatory sequence.

For example, a selectable marker cassette comprising the nos promoter and nos terminator regulatory sequences controlling the expression of thenptII gene was PCR-amplified using a proofreading DNA polymerase from the binary vector pKYLX71: 35S2 and directionally cloned into the AgeI and NotI sites of the auxiliary vector pAUX3166. Equally, other selectable marker cassettes can be introduced into any of the auxiliary vectors.

In another example, the expression cassette from the binary vector pWM5 consisting of the ASSU promoter and the tob terminator was PCR-amplified with a proofreading DNA polymerase and directionally cloned into the AgeI and NotI sites of the auxiliary vector pAUX3169. Equally, other expression cassettes can be introduced into any of the auxiliary vectors.

In yet another example, the expression cassette from the direct gene transfer vector pDH51 was cut usingEcoRI and cloned directly into theEcoRI site of the auxiliary vector pAUX3132.

List of primers used to PCR-amplify plant selectable marker cassettes and the regulatory elements used to control the expression of CS, MDH and PEPC genes

column inserted

expression cassette	primer	Seq. ID No.	primer sequence (5'>3')
nos::nptII-nos	forward	387	ATAATAACCGGTTGATCATGAGCGGAGAATTAAGGG
	reverse	388	ATAATAGCGGCCGCTAGTAACATAGATGACACCGCG
35S::aacC1-35S	forward	389	AATAGCGGCCGCGATTTAGTACTGGATTTTGG
	reverse	390	AATAACCGGTACCCACGAAGGAGCATCGTGG
$35S^2$::rbcS	forward	391	ATAATAACCGGTGCCCGGGGATCTCCTTTGCC
	reverse	392	ATAATAGCGGCCGCATGCATGTTGTCAATCAATTGG
assu::tob	forward	393	TAATACCGGTAAATTTATTATGRGTTTTTTTCCG
	reverse	394	TAATGCGGCCGCTAAGGGCAGCCCATACAAATGAAGC

The expression cassettes were further modified by introducing a GATEWAY® cloning cassette (Invitrogen) into the multicloning site of the respective pAUX vector following the manufacturer's protocol. In a recombination reaction, the cDNAs encoding the open reading frame sequences were transferred from the entry vector obtained as described in Example 4 to the GATEWAY®-enabled pAUX vector. Any combination of the regulatory elements with cDNA sequences of TrCSa, TrCSb, TrCSd, TrMDH and TrPEPC can be obtained. One typical example is given in Figure ~~126~~ 34 with expression cassettes comprising the nptII plant selectable marker, TrPEPC, TrCSa and TrMDH.

Complete expression cassettes comprising any combination of regulatory elements and cDNA sequences to be introduced into the plant were then cut from the auxiliary vectors using the respective homing endonuclease and cloned into the respective restriction site on the binary vector backbone. After verification of the construct by nucleotide sequencing, the binary transformation vector comprising a number of expression cassettes was used to generate transgenic white clover plants.

EXAMPLE 6

Isolation of regulatory elements to direct expression of chimeric genes encoding CS, MDH and PEPC involved in organic acid biosynthesis

To direct the expression of chimeric white clover genes TrCSa, TrCSb, TrCSd, TrMDH and TrPEPC involved in organic acid biosynthesis to specific tissues, regulatory elements showing specificity for expression in root or root tip tissue were identified and isolated.

Using the BLASTn algorithm, white clover EST sequence collections prepared as detailed in Examples 1 and 2 were searched with nucleotide sequences representing genes with known root-specific expression identified in GenBank as queries. Suitable candidate ESTs were identified and oligonucleotide primers for reverse transcription-PCR (RT-PCR) were designed (see Table 4).

TABLE 6

Oligonucleotide primers used in reverse transcription-PCR to confirm tissue specificity of candidate white clover ESTs

gene	forward primer (5'->3')	reverse primer (5'->3')
histone (internal control)	CCGATTCCGTTTCAATGGCTCGTA SEQ ID No: 395	GCCATCCTTAACCCTAAGCACGT SEQ ID No: 396
white clover phosphate transporter homolog	TTGCATTTGCTTGGAACAACTAG SEQ ID No: 397	GCAAGAGCAAACATGAAACCA SEQ ID No: 398
white clover root iron transporter homolog	ATGGGTCTTGGTGGTTGCA SEQ ID No: 399	GCAGCAAGAAGATCAACCAAAGCCA SEQ ID No: 400

Seq ID Nos inserted

Total RNA for RT-PCR experiments was isolated from a leaf, stolon, stolon tip, root and root tip of white clover plants grown in the glasshouse using the TRIZOL method. Reverse transcription was performed using SuperScriptII (Invitrogen) following the supplier's instructions. Preliminary PCR reactions using Dynazyme as the DNA polymerase were set up to determine the correct amount of template using the PCR primers for the internal control (histone). The results of this preliminary PCR were used to set up another round of PCR to determine the optimum number of cycles for linear amplification. The final PCR amplifications were performed using the following cycling conditions: 94 ºC, 4 min., 1 time; 94 ºC, 15 sec., 60 ºC, 30 sec., 72 ºC, 2 min., x times; 72 ºC, 10 min., 1 time. The number

were performed using the following cycling conditions: 94 C, 4 min. , 1 time; 94 C, 15 sec. , 60 C, 30 sec. , 72 C, 2 min. , x times; 72 C, 10 min. , 1 time. The number of cycles in the amplification (x) was found to be dependent on the relative abundance of transcript and had to be optimised for each template.

RT-PCR results using a white clover histone gene as an internal constitutively expressed control confirmed the tissue-specificity of two candidate ESTs to be root-prevalent (Figure ~~127~~ 35 A and B). These were a phosphate transporter homolog (clone name02wc1 DsG07) and a root iron transporter homolog (clone name05wc1 IsB11).

A spotted white clover BAC library consisting of 50,304 clones with an estimated 99% genome coverage (6.3 genome equivalents) was screened using the phosphate transporter homolog EST nucleotide sequence as a probe. A number of positive BAC clones could be identified (Figure ~~128~~ 36 A). After Southern hybridisation blotting (Figure ~~128~~ 36 B) a 7.5 kb EcoRV genomic DNA fragment was selected and fully sequenced. The fragment contained the phosphate transporter homolog open reading frame and 4 kb of upstream sequence including the promoter region. A physical map of the genomic DNA fragment including the promoter region is shown in Figure ~~128~~ 36 C.

EXAMPLE 7

Production by Agrobacterium-mediated transformation and analysis of transgenic white clover plants carrying chimeric genes encoding CS, MDH and PEPC involved in organic acid biosynthesis

A set of binary transformation vectors carrying chimeric white clover genes to alter the expression of the polypeptides involved in organic acid biosynthesis to improve phosphorus acquisition efficiency as well as aluminium and acid soil tolerance in forage plants were produced as detailed in Examples 4 and 5.

Agrobacterium-mediated gene transfer experiments were performed using these transformation vectors.

The production of transgenic white clover plants carrying the white clover TrCSa, TrCSb, TrCSd,TrMDH andTrPEPC cDNAs, either singly or in combination, is described here in detail (Table 7).

Preparation of genomic DNA and analysis of DNA for presence and copy number of transgene by Southern hybridisation blotting

Genomic DNA for Southern hybridisation blotting was obtained from leaf material of white clover plants following the CTAB method. Southern hybridisation blotting experiments were performed following standard protocols as described in Sambrook etal. (1989). In brief, genomic DNA samples were digested with appropriate restriction enzymes and the resulting fragments separated on an agarose gel. After transfer to a membrane, acDNA fragment representing a transgene or selectable marker gene was used to probe the size-fractionated DNA fragments. Hybridisation was performed with either radioactively labelled probes or using the non-radioactive DIG labelling and hybridisation protocol (Boehringer) following the manufacturer's instructions.

Plants were obtained after transformation with all chimeric constructs and selection on medium containing gentamycin. Details of plant analysis are given in Table 5 and Figures ~~130~~38,~~131~~ 39 and ~~132~~40.